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FINAL TECHNICAL REPORT
LIFE SCIENCES NAGW-0097

LOCALIZE AND IDENTIFY THE GRAVITY SENSING
MECHANISM OF PLANTS

I. Description of research:

Our intent is to localize and identify, at the cellular level, the machinery by means of which a plant transduces the gravity stimulus into a growth response. To accomplish this difficult task we adopted a reductionist approach and separated the plants gravity response into: a) perception; b) transduction; and c) asymmetric growth.

We early decided not to study, c) asymmetric growth, since growth is too complex a subject for attaining a molecular level understanding of the tropic response. Progress in: a) perception and b) transduction; seemed possible. However even here a further reduction of scope seemed necessary. The fact that a plant grows unequally on the lower side of a horizontally placed stem means that there must be asymmetric distribution of some of the chemical substances involved in the growth response. Thus, it is possible to rephrase the question of transduction into the simpler question: how can a plant attain an asymmetric distribution of one of the chemicals involved in growth? The three most prominent and likely chemical contenders were potassium, calcium, or, the growth hormone, indole-3-acetic acid (IAA). This laboratory had two decades of experience in the analysis, chemistry, and metabolism of IAA and its adducts and so the asymmetric distribution of IAA was a reasonable choice (1).

Under results we will describe how this question of-- how does a plant attain an asymmetric distribution of IAA-- has led us to a fairly complete understanding of the transduction of the gravity stimulus (b above) and led to an interesting surmise regarding gravity perception (a above).

II. Accomplishments: (References 1 to 12 are listed at the end of this section. Higher numbers refer to publications from this laboratory and are listed under Publications at the end of this report.

a) Potential-gating theory: We must treat this accomplishment first since much of what follows has been guided by this theory. We knew that the sequence of events following a gravity stimulus was (172,184,192,203,212,223, 233,240,242,253):

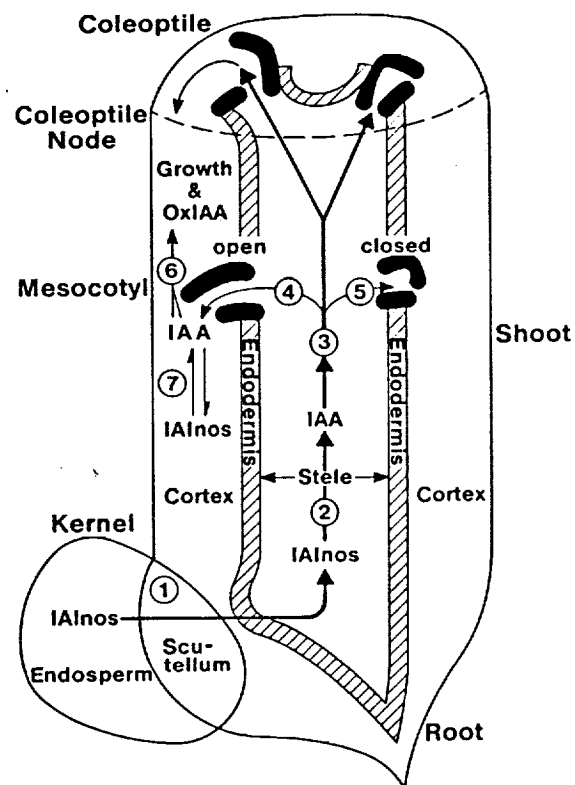


Fig. 1. A diagrammatic representation of the Potential Gating Theory showing the postulates of how an asymmetric distribution of IAA might occur following a tropic stimulus. The mechanism emphasizes transport of IAA and not de novo synthesis of IAA and encompasses portions of the original Went-Cholodny theory. (1) Indole-3-acetyl-*myo*-inositol (IAInos), the storage and transport form of IAA in *Zea mays*, would leave the endosperm and enter the vascular stele of the mesocotyl. (2) At some point in the stele, the IAAInos is hydrolyzed to IAA. (3) The IAA and/or IAAInos moves to the coleoptile tip and the coleoptile-mesocotyl node. (4),(5) The central stele has no apoplastic communication with the surrounding cortical cells and the only means of egress of IAA from the stele and into the cortex plus epidermis is through the plasmodesmata. The channels in the plasmodesmata are gated by the membrane potential into an open position (4) or a closed position (5). The so-called lateral migration of IAA would be attained by selective movement through the open channel. (6) In the cortex plus epidermis, the IAA commits the growth promoting act and is, in turn, oxidized to oxindole-3-acetic acid (OxIAA). (7) IAA not used immediately in growth may be reconstituted as an IAA ester, such as IAAInos, that is not active in growth promotion but may later be hydrolyzed to yield free IAA. Asymmetric growth, characteristic of the tropic response would ensue owing to the asymmetric distribution of IAA and to the subsequent, or simultaneous, cascade of ion movements leading to growth.

- i) gravity stimulus>>>
- ii) membrane depolarization (within 8 sec)>>>
- iii) asymmetric distribution of IAA (within 3 min)>>>
- iv) asymmetric distribution of calcium (about 5 min)>>>
- v) asymmetric growth (within 10 minutes)>>>

The theory resulted from asking the question--how can an electrical perturbation, such as a membrane depolarization, ii) above, result in an asymmetric distribution of a chemical such as IAA (iii above)? Briefly stated the theory is (212,233,253):

- 1) The gravity stimulus is perceived by an unspecified mechanism;
- 2) The stimulus causes membrane depolarization;
- 3) Membrane depolarization results in opening and/or closing transport channels between the conductive tissue and the target tissue;
- 4) IAA flows through the open channels into the cortical tissue target and is prevented from moving through closed channels;
- 5) The asymmetric hormone distribution causes asymmetric growth and thus the righting response;
- 6) The plant grows back into its normal vertical orientation and free IAA levels returned to normal.

Figure 1 (253) presents a diagrammatic representation of the Potential-Gating Theory. A test of this theory is underway as part of a flight experiment NAG 2-362.

b) Asymmetric (free plus ester) IAA distribution:

Mrs. Aga Schulze early discovered (219) that the vascular stele of a young corn seedling could easily be removed from the mesocotyl leaving pure cortical tissues uncomplicated by vascular conductive tissue. We also early found that the stele contained mainly free IAA whereas the cortex contained mainly ester IAA (2, 262). Mrs. Schulze also found that both free and ester IAA (that is the total IAA of the cortex became asymmetrically distributed in the cortex within 3 min following the gravity stimulus (184,240). We had early postulated that the gravity stimulus caused a change in ratio of free to ester IAA thus resulting in more free IAA (3). We had to discard this postulate since both free and ester IAA increased in the lower cortex of a horizontally-placed plant (eg. 240,253). A change in the distribution of total IAA had to involve either transport of IAA from a source such as the seed, or de novo synthesis of IAA.

c) De novo synthesis of IAA:

To distinguish between transport and/or de novo biosynthesis of IAA to account for the "extra" IAA in the lower cortex of a horizontally placed seedling it was necessary to determine whether de novo biosynthesis of IAA did in fact

occur. To test for de novo aromatic biosynthesis we chose a method that would detect IAA synthesis without prejudice as to the biosynthetic route. Kernels were imbibed in and grown on 30% D₂O. If the indole ring of IAA was synthesized deuterium would be incorporated into the indole ring and this could be detected by mass spectrometry. Mr. Philip Jensen performed this experiment with the results shown in Table I (225,238,247). As can be seen, there is no deuterium incorporated into IAA during the 7 days of this experiment. By contrast there is incorporation of deuterium into tryptophan. Subsequent studies by means of high field NMR (247) showed the deuterium to be present in position 6 of the benzenoid ring and possibly in 5 and 7 but not in 4.

symmetry.

TABLE I Incorporation of deuterium into indole-3-acetic acid and tryptophan by *Zea mays* grown on H₂O or 30% D₂O for 7 days in the dark. M/z=130 is base peak for both methyl IAA and methyl monoacetyl tryptophan, the two derivatives used here. It represents the quinolinium ion resulting from cyclization of the methyl indole fragment.

Percentage of molecules at the indicated mass		
	Control	30% D ₂ O
Tryptophan		
m/z 130	89.9	43.8
m/z 131-136	10.1	56.2
IAA		
m/z 130	89.9	90.0
m/z 131-136	10.1	10.0

We conclude that tryptophan is being biosynthesized in a 7 day old germinating corn seedling but IAA is not being synthesized. Thus, de novo synthesis of IAA cannot account for the IAA asymmetry.

d) Asymmetric transport of IAA:

It was important to visualize the seedling plants as separated into transport domains and to understand how the domains were connected. The first such attempt (194,197,204) was made by Mrs. Schulze utilizing the polyanionic sulfonic acid dye, light green. So strong an acid could never be protonated at a physiological pH and thus would be excluded from the symplast and be confined to apoplastic space for transport. Professor Bernard Epel made further, more detailed, studies and concluded that transport of dye through the mesocotyl was apoplastic and since none of the dye moved from stele to cortex there could be no apoplastic communication between stele and cortex. By contrast, acetyl carboxyfluorescein (a known symplast indicator), and IAA move from stele to cortex, IAA with greater facility than carboxyfluorescein (249,250 and In Press). Epel concluded there was no apoplastic communication between the stele and cortex of the mesocotyl. Since the interface between stele

and cortex is suberized. Thus, the only communication between stele and cortex are the plasmodesmata which connect stele and cortex through the endodermal barrier.

We conclude that the target of the gravity stimulus must be some sort of gating mechanism located in the plasmodesmata that connect the vascular stele to the cortex plus epidermis that surrounds the stele.

e) The potential difference between stele and cortex:

There are innumerable studies of bioelectric potentials in plant tissues dating back to Cholodny (4), Brauner (5), Lund (6) and Tanada (7). However, it was the recent studies of the Siever's group (8) examining membrane potentials which attracted our attention. They had shown a marked change in membrane potential within 8 seconds after a positional change in a plant root. It was this observation, and particularly the work of Tanada, which made us ask the question: How can an electrical potential cause an asymmetric distribution of a chemical compound? Clearly, it was not simply an electrophoretic movement of compound since Momonoki in our laboratory had shown that not only IAA but IAA ester and even glucose-the two later being non-charged, neutral compounds not subject to electrophoretic migration, became asymmetrically distributed (232A). We knew of the existence of voltage-gated gap junctions (9) and it was this knowledge that led us to postulate that the transport channels for IAA in the plasmodesmata passing from stele to cortex were voltage-gated (203,204,212,240,253).

Dr. Mark Desrosiers began a series of experiments attempting to determine the relationship between the potential of the stele and the potential of the IAA target cells lying in the cortex and surrounding epidermis. He early determined that a small controlling voltage of only 0.6mV per cell with the wrong polarity could stop the growth of cells having an endogenous membrane potential of 100mV per cell. Thus, as is shown in Fig.2 five volts applied to an 8 cm section of tissue inhibits growth 90% if the tip of the plant is made positive relative to the roots and causes no inhibition if the tip of the plant is made negative (229).

A striking chemical observation was made by Schulze and Desrosiers in that this same small controlling voltage caused an increase in ester IAA accumulating in the stele (228). If IAA can not leave the stele and enter the cortical cells, it is possible this could result in the growth inhibition observed.

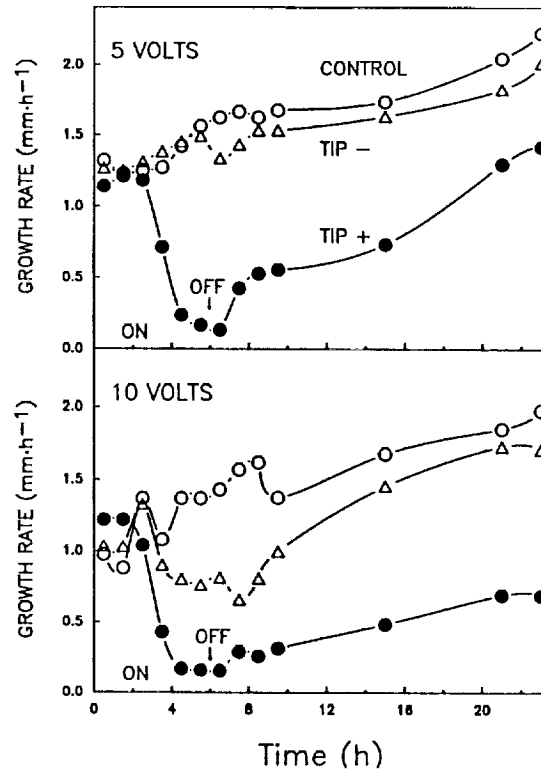
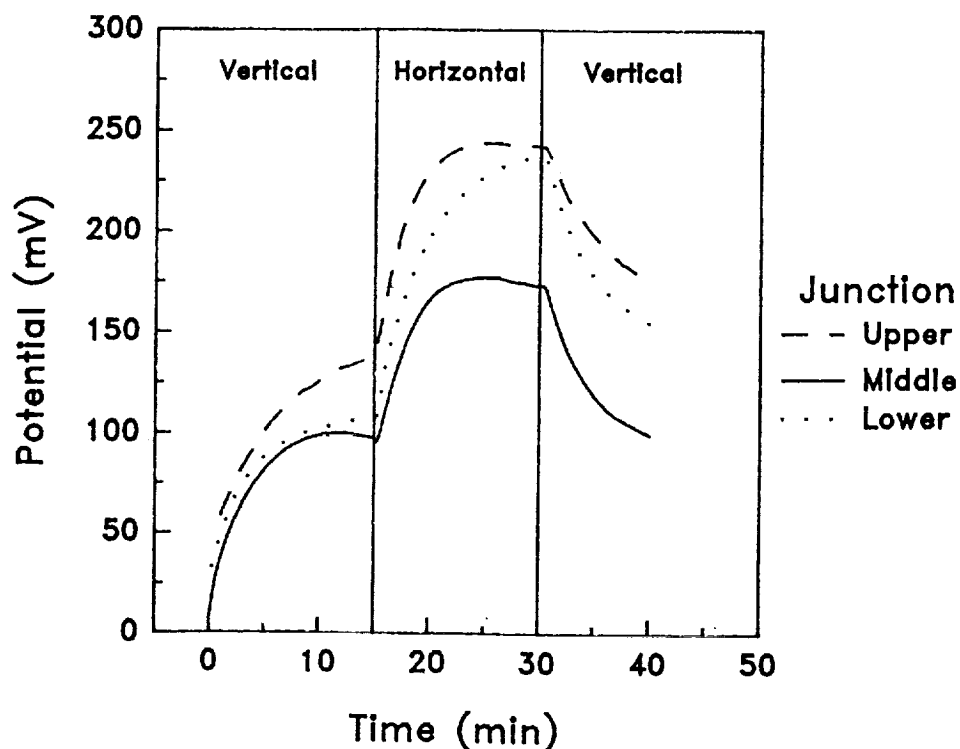


FIG. 2 Growth rate in millimeters per hour of 4-d, dark-grown corn seedlings' shoots as a function of time and of the magnitude and polarity of a voltage applied for 4 h. The electrical potential was applied between 2 and 6 h to the shoots with the apical tip at negative (Δ) or positive (\bullet) potential with respect to the base of the shoot or with no potential applied, control (O). Each point is the average of 30 independent measurements.

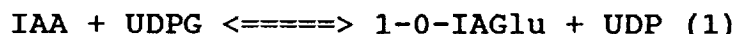
Most recently, Dr. Desrosiers has studied the potential difference between the cells in the stele and the cells in the cortical tissues (255). For this purpose a platinum electrode is inserted into the stele and a second platinum electrode inserted into the cortical tissues. By means then of a very high quality electrometer it was possible to show that a change in the orientation of a small piece of stem tissue from a vertical orientation to a horizontal one was enough to cause changes in potential difference of almost 150 mV as shown in Fig. 3.

We conclude that the effects of an applied potential as well as changes in endogenous potential between stele and cortex are those predicted from the potential gating theory.

Figure 3

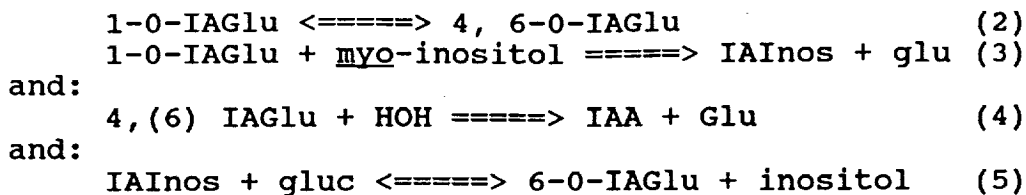


f) There is lastly the matter of the metabolism, and particularly esterification, of IAA in the cortical tissues and the source of the free IAA in the stele. To this end we have continued our studies of the metabolism of IAA financed primarily by NSF funds. Dr. Kowalczyk and Mr. Maciej Pawlak have purified the enzyme catalyzing the synthesis of 1-0-IAA-glucose to homogeneity and prepared antibodies to this enzyme (230,231,251). Since IAGlu is the first compound in the series of esters formed in Zea mays seedlings, the enzyme catalyzing this reaction is likely to be a control point enzyme. The reaction catalyzed is:



It is our intention to clone this enzyme and ultimately to prevent the expression of this reaction so as to determine the effects of the failure of a plant to synthesize ester conjugates and thus control levels of free IAA.

Of equal interest has been the reactions that lead to the production of free IAA from the IAA-myo-inositol found in the kernel. Dr. Kowalczyk, Mr. Jacek Kesy, and Mr. Maciej Pawlak have found the following reactions (244,245,251):



These reactions, acting in consort are capable of providing the shoot with a source of IAA by transport and hydrolysis of IAINos since IAINos is a major storage form of IAA in the kernels. Of possible physiological significance is the fact that the enzyme catalyzing reaction (1) cofractionates with enzymes catalyzing the hydrolysis of both 1-0-IAGlu and 6-0-IAGlu as shown in Fig. 4.

We conclude that the enzymes found and characterized in this laboratory will catalyze the reactions necessary to supply IAA to the young growing shoot and to restore hormonal balance following the tropic response.

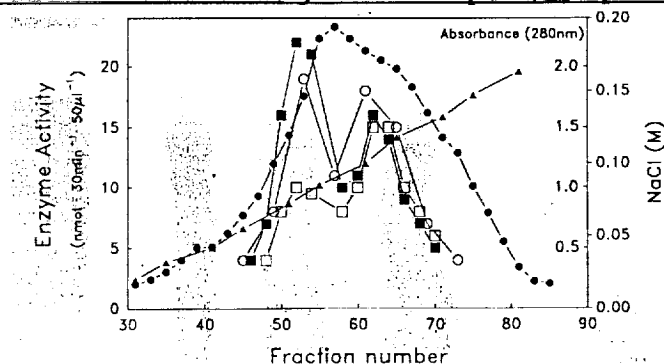


Fig. 4. Chromatography on DEAE-Sephacel of the IAGlu hydrolase and IAGlu synthase not bound to a Blue-Sepharose column. The Proteins not bound to Blue Sepharose comprised the bulk of IAGlu hydrolase activity and were further separated on a DEAE-Sephacel column using a gradient of 0 to 0.2 M NaCl (▲). IAGlu hydrolase activity was determined by measuring the appearance of free IAA (□) or free glucose (■). IAGlu synthase activity (○) and proteins (●) were measured as described in "Materials and Methods."

g) Perception of the gravity stimulus:

Our work has not been directly concerned with the mechanism by means of which a plant can detect the gravity vector. However, owing to some work by Professor Christos Summerville in this department, some important conclusions can be made which are relevant to the Potential Gating Theory. Casper and Summerville (10) prepared a mutant of Arabidopsis lacking the enzyme phosphoglucomutase in the chloroplasts. Thus, this mutant can not produce the dense amyloplasts allegedly involved in gravity detection. The surprising thing is that the mutant is almost normal with respect to the gravity response. There has been some dispute as to whether the response is normal or only nearly normal (11) but that really misses the point that a plant lacking dense starch grains can still respond to the gravity

stimulus. We must conclude that plants have multiple gravity detecting mechanisms and, of course, this is not surprising. Both light and gravity are all pervasive stimuli and one might expect multiple detection mechanisms for gravity just as there are multiple light detection mechanisms.

So we may surmise the following mechanisms for the detection of gravity by plants:

1) a falling heavy body such as a statolith may be used if available;

2) stretch activated channels such as studied by Pickard (12) and postulated in the Solion model previously presented by this laboratory (194,212);

3) we postulate a direct interaction between the gravity field and the dipole of the plant (212). If one visualizes each cell of the plant as a small dipole and then one envisages each small dipole to be stacked so as to make a large, highly polarized structure, it seems possible that the movement of this structure in the gravity and the geomagnetic fields of the earth may itself serve as a gravity sensing mechanism.

We conclude that the plant possesses several gravity sensing systems any one of which can lead to membrane depolarization and the resultant gating that leads to an asymmetric distribution of the plant growth hormone, IAA.

III. Significance of the accomplishments:

The significance is really three fold:

First, we have learned a great deal concerning the mechanisms by means of which a plant can control the amount of growth hormone (IAA) in its tissues. This has been an objective of this laboratory for many years and we feel it will be of great importance in agriculture as more subtle means of plant growth control by means of growth regulating chemicals are devised. If we know how a plant controls hormone levels then we should be able to control those same control mechanisms.

Second, the ability to grow plants in space will benefit by knowledge of the plants control mechanisms. From the experiments provided here and those provided by our flight experiment, we are reasonably certain that the plant is plastic enough to do well in micro-gravity providing only that a substitute vector for the gravity vector can be provided. Probably a potential field should serve in lieu of gravity for orientation of root growth.

Third, we may learn something of gravity itself. Certainly we are learning how gravity interacts with a living organism.

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IV. Publications:

(The following publications were supported wholly, or in part, by NAGW-0097 during the period August 1, 1980 to October 31, 1989).

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